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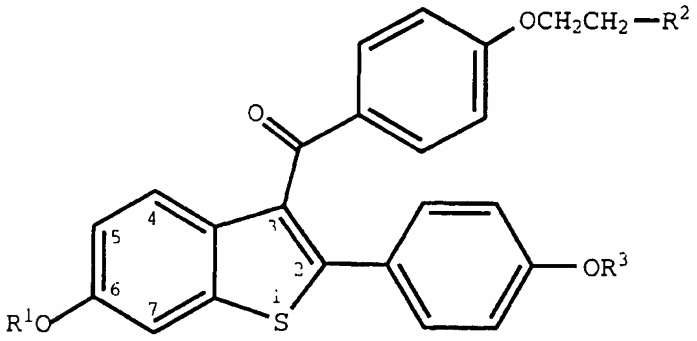
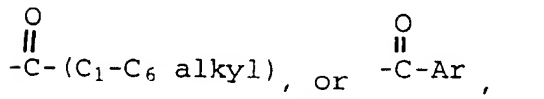
(54) 2-Phenyl-3-azoylbenzothiophenes for treating male infertility.

(57) A method of inhibiting male infertility comprising administering to a human in need thereof an effective amount of a compound having the formula



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$,



wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidine, hexamethyleneamino, and piperidino; or a pharmaceutically acceptabl salt of solvat thereof.

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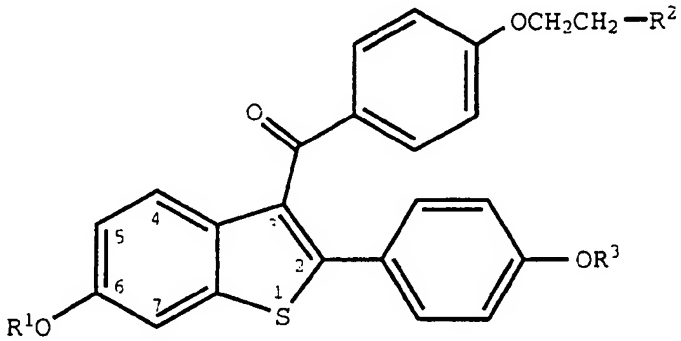
An estimated one in five couples in the United States has some degree of infertility. Infertility is defined as the inability of a heterosexual couple to achieve a pregnancy within one year of unprotected intercourse (Cecil Textbook of Medicine, W.B. Saunders Company, 19th Ed., p. 1339-1340, (1992)). Major etiological factors include ovulatory dysfunction, abnormal tubal function, cervical factors, and male sperm factors. (The Merck Manual of Diagnosis and Therapy, Merck Research Laboratories, 16th Ed., p. 1768-1770, (1992)). An estimated five to six percent of men in the reproductive age group are infertile. Most causes of male infertility are due to an abnormal sperm count or low semen quality.

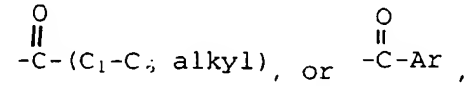
A majority of problems associated with fertility in males stem from changes in testosterone levels. In particular, decreases in concentration of this steroid can result in infertility and impotence. Endogenous estrogen has been well-documented to serve as a regulatory factor in testosterone production by interaction with the estrogen receptor (Nozu, K. et al., J. Biol. Chem. 256, 1915 (1981); Brinkman, A. et al., Endocrinology, 110, 1834 (1982)). Thus, intratesticular estrogen plays a key role in testosterone steroidogenisis with increased levels of estrogen inhibiting testosterone production (Cigorraga, S.B. et al., J. Clin. Invest. 65, 699 (1982); Padron, R.S.J., Clin. Endocrinol. Metab. 50, 1100 (1980)). A need exists for new methods of treating or preventing male infertility.

This invention provides methods for inhibiting male infertility comprising administering to a human in need thereof an effective amount of a compound of formula I



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$,



wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidino, hexamethyleneimino, and piperidino; and pharmaceutically acceptable salts and solvates thereof.

The current invention concerns the discovery that a select group of 2-phenyl-3-aroylbenzothiophenes (benzothiophenes), those of formula I, are useful for inhibiting male infertility. The methods of treatment provided by this invention are practiced by administering to a human in need thereof a dose of a compound of formula I or a pharmaceutically acceptable salt or solvate thereof, that is effective to inhibit male infertility. The term inhibit is defined to include its generally accepted meaning which includes prophylactically treating a human subject to incurring the problem described, and holding in check and/or treating an existing problem. As such, the present method includes both medical therapeutic and/or prophylactic treatment, as appropriate.

Raloxifene, a preferred compound of this invention, is the hydrochloride salt of a compound of formula 1, wherein R^1 and R^3 are hydrogen and R^2 is 1-piperidinyl, and is a nuclear regulatory molecule. Raloxifene has been shown to bind to the estrogen receptor and was originally thought to be a molecule whose function and pharmacology was that of an anti-estrogen in that it blocked the ability of estrogen to activate uterine tissue and estrogen dependent breast cancers. Indeed, raloxifene does block the action of estrogen in some cells; however in other cell types, raloxifene activates the same genes as estrogen does and displays the same pharmacology, e.g., osteoporosis, hyperlipidemia. The unique profile which raloxifene displays and differs from

that of strogen is now thought to be due to the unique activation and/or suppression of various gen functions by th raloxifene-estrog n receptor complex as opposed to th activation and/or suppression of g nes by th estrog n-estrogen receptor complex. Th refore, although raloxifene and strogen utilize and compete for the same receptor, the pharmacological outcome from gene regulation of the two is not easily predicted and is unique to each. It is b li v d that the compounds described herein act to block the inhibitory properties of estrogen on testosterone production.

Generally, the compound is formulated with common excipients, diluents or carriers, and compressed into tablets, or formulated as elixirs or solutions for convenient oral administration, or administered by the intramuscular or intravenous routes. The compounds can be administered transdermally, and may be formulated as sustained release dosage forms and the like.

The compounds used in the methods of the current invention can be made according to established procedures, such as those detailed in U.S. Patent Nos. 4,133,814, 4,418,068, and 4,380,635 all of which are incorporated by reference herein. In general, the process starts with a benzo[b]thiophene having a 6-hydroxyl group and a 2-(4-hydroxyphenyl) group. The starting compound is protected, acylated, and deprotected to form the formula I compounds. Examples of the preparation of such compounds are provided in the U.S. patents discussed above. Optionally substituted phenyl includes phenyl and phenyl substituted once or twice with C_1-C_8 alkyl, C_1-C_4 alkoxy, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)methyl.

The compounds used in the methods of this invention form pharmaceutically acceptable acid and base addition salts with a wide variety of organic and inorganic acids and bases and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanoic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, β-hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, teraphthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzene-sulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate and the like. A preferred salt is the hydrochloride salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

Bases commonly used for formation of salts include ammonium hydroxide and alkali and alkaline earth metal hydroxides, carbonates, as well as aliphatic and primary, secondary and tertiary amines, aliphatic diamines. Bases especially useful in the preparation of addition salts include ammonium hydroxide, potassium carbonate, methylamine, diethylamine, ethylene diamine and cyclohexylamine.

The pharmaceutically acceptable salts generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Pharmaceutical formulations can be prepared by procedures known in the art. For example, the compounds can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium st arate, and solid polyethyl glycols.

Th compounds can also be formulat d as elixirs r solutions for conveni nt oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutan ous or intravenous routes. Additionally, the compounds ar well suited to formulation as sustain d release dosage forms and th

like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

The particular dosage of a compound of formula I required to inhibit male infertility according to this invention will depend upon the severity of the condition, the route of administration, and related factors that will be decided by the attending physician. Generally, accepted and effective daily doses will be from about 0.1 to about 1000 mg/day, and more typically from about 50 to about 200 mg/day. Such dosages will be administered to a subject in need of treatment from once to about three times each day, or more often as needed to effectively treat the problem.

It is usually preferred to administer a compound of formula I in the form of an acid addition salt, as is customary in the administration of pharmaceuticals bearing a basic group, such as the piperidino ring. It is also advantageous to administer such a compound by the oral route. For such purposes the following oral dosage forms are available.

Formulations

In the formulations which follow, "active ingredient" means a compound of formula I.

Formulation 1: Gelatin Capsules

Hard gelatin capsules are prepared using the following:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.1 - 1000
Starch, NF	0 - 650
Starch flowable powder	0 - 650
Silicone fluid 350 centistokes	0 - 15

The ingredients are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules.

Examples of specific capsule formulations of the compound of formula 1 wherein R^2 is piperidino, (raloxifene), that have been made include those shown below:

Formulation 2: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	1
Starch, NF	112
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 3: Raloxifene capsul

Ingr di nt	Quantity (mg/capsule)
Raloxifene	5
Starch, NF	108
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 4: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	10
Starch, NF	103
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 5: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	50
Starch, NF	150
Starch flowable powder	397
Silicone fluid 350 centistokes	3.0

The specific formulations above may be changed in compliance with the reasonable variations provided.
A tablet formulation is prepared using the ingredients below:

Formulation 6: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Cellulose, microcrystalline	0 - 650
Silicon dioxide, fumed	0 - 650
Stearate acid	0 - 15

The components are blended and compressed to form tablets.
Alternatively, tablets each containing 0.1 - 1000 mg of active ingredient are made up as follows:

Formulation 7: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60° C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.1 - 1000 mg of medicament per 5 mL dose are made as follows:

Formulation 8: Suspensions

Ingredient	Quantity (mg/5 ml)
Active ingredient	0.1 - 1000 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to	5 mL

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

ASSAYS

Assay I

The following assay is described in Cigorraga *et al., J. Clin Invest, 65,* 699-705, March 1980, incorporated herein by reference.

Five to fifty male rats (200-250g) are obtained. Gonadotropin-induced desensitization of Leydig cells is achieved by intravenous injection of hCG or by subcutaneous injection of GnRH. A compound of formula 1 is administered with the intravenous hCG dose or alone in controls and before subcutaneous administration of LH r leasing-hormone. Animals are killed by decapitation 2 or 3 days after injection of gonadotropin or GnRH, and interstitial cells from test s of normal and treated animals ar prepared by collag nase dig stion. The cells are further fractionated by density gradient centrifugation, giving purified cell preparations containing ov r 90% L ydig cells as judged by morphological criteria and metabolic responses. The purified Leydig cells are

washed once and resuspend d in a medium containing 0.1% bovin s rum albumin. Th proportion of incubation m dium to cells is quivalent to 2 ml/testis, giving about 10^6 purified L ydig cells/ml.

L ydig cells are incubat d with purified hCG or dibutyryl cyclic (c)AMP (Bt_2cAMP). When pregn nolone accumulation is to be measured, inhibitors of 3β-hydroxyst roid dehydrogenase and 17-hydroxylase are added to cell incubations before the addition of stimuli; control incubations ware treated similarly.

Groups of rats are also studied after the following treatments (a) control; (b) intravenous injection of hCG; (c) intravenous injection of hCG plus a compound of formula 1 and i.m.; *(d)* subcutaneous injections of hCG. The rats are killed at selected times ater the injections. Blood samples collected from the decapitated animals are allowed to clot, and serum is stored at -70°C before testosterone analysis. Testes are removed and kept frozen until analyzed for estradiol 17β, testosterone, progesterone, and 17α-hydroxyprogesterone.

*Assays of steroids and serum h*CG.

Decapsulated testes are homogenized in PBS/testis and extracted with ethyl acetate after addition of tracer amounts of H-steroids to account for losses during the fractionation procedure. Testosterone is measured and the testosterone content of testis extracts and serum is determined after isolation of the steroid. Pregnenolone is measured with a highly specific rabbit antiserum to the 11-hemisuccinate albumin conjugate. Radioimmunoassay of 17α-hydroxyprogesterone is performed with an antiserum to the 3-carboxymethyloxime derivative. 17β-estradiol assays are performed using a highly specific rabbit antiserum to 6-Ketoestradiol conjugated to bovine serum albumin. Immunoreactive serum hCG concentrations are measured.

Assay of LH receptors in dispersed Leydig cells.

Radioiodinated hCG tracer is prepared by lactoperoxidase method and purified by sepharose-concanavalin A chromatography. Purified Leydig cells (5×10^5) are incubated for 3 h at 34°C with 5×10^5 dpm of ^{125}I-hCG (Specific activity 40 μCi/μg) with additions of hCG to ensure receptor saturation. Nonspecific binding is determined by incubation of cells with the labeled hormone in the presence of unlabeled hCG. All binding capacities are calculated for replicate estimations of specific ^{125}I-hCG binding at saturation, with corrections for specific activity and maximum bindability of the tracer preparation. The mean binding capacity is calculated for each of the experimental groups and expressed as a percentage of control values, or as the number of receptor sites per cell.

Increases in the cellular LH receptors and/or testosterone responses, or prevention of reduction of maximal testosterone response in Leydig cells from hCG-desensitized animals, illustrate the activity of the compounds of formula 1.

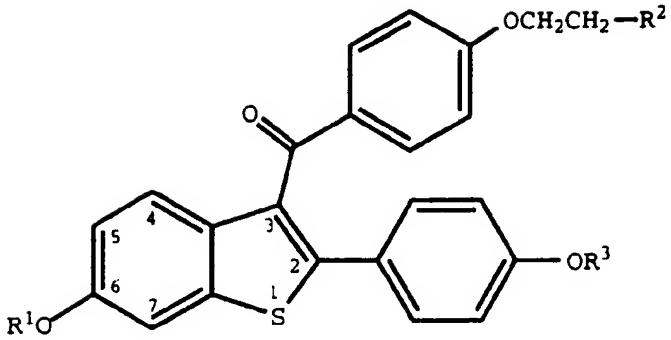
Assay II

Five to fifty men are selected for the clinical study. The men are are in good general health, but suffer from infertility. The study has a placebo control group, i.e., the men are divided into two groups, one of which receives the active agent of this invention and the other receives a placebo. All men in the study have their sperm benchmarked for quality and quantity. Men in the test group receive between 50-200 mg of the active agent per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the benchmarks in both groups and at the end of the study these are compared. The results are compared both between members of each group and also the results for each patient are compared to the benchmarks of each patient before the study began.

Utility of the compounds of the invention is illustrated by the positive impact they have in at least one of the above assays.

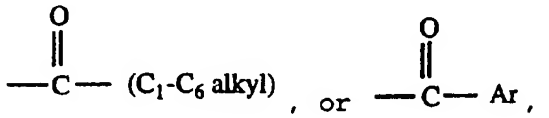
Claims

1. The use of a compound having the formula



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$,



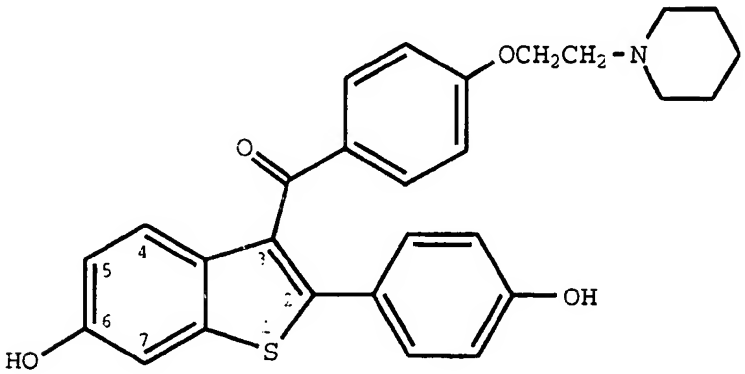
wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidino and piperidino; or a pharmaceutically acceptable salt or solvate thereof, in the preparation of a medicament useful for inhibiting male infertility.

2. The use of Claim 1 wherein said compound is the hydrochloride salt thereof.

3. The use of Claim 1 wherein said medicament is prophylactic.

4. The use of Claim 1 wherein said compound is



or its hydrochloride salt.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 9482

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,Y	US-A-4 418 068 (JONES) 29 November 1983 * abstract; claims * ---	1-4	A61K31/445 A61K31/40 A61K31/38
Y	JOURNAL OF MEDICINAL CHEMISTRY, vol. 27, no.8, 1984 pages 1057-1066, C.D. JONES ET AL. 'ANTIESTROGENS.2.' * the whole document * ---	1-4	
Y	HORMONE RESEARCH, vol. 28, no.2-4, 1987 pages 219-229, J. BUVAT ET AL. 'ANTIESTROGENS AS TREATMENT OF FEMALE AND MALE INFERTILITIES' * the whole document * ---	1-4	
Y	ANDROLOGIA, vol. 19, no.1, 1987 pages 86-90, D.I. LEWIS-JONES ET AL. 'IMPROVEMENT IN SEMEN QUALITY IN INFERTILE MALES AFTER TREATMENT WITH TAMOXIFEN' * the whole document * -----	1-4	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23 March 1995	Hoff, P

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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